*Intern. J. Neuroscience*, 2000, Vol. 100, pp. 107–116
Reprints available directly from the publisher
Photocopying permitted by license only

© 2000 OPA (Overseas Publishers Association) N.V.
Published by license under
the Gordon and Breach Science
Publishers imprint.
Printed in Malaysia.

# SUPPRESSION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN RATS EXPOSED NOCTURNALLY TO MAGNETIC FIELDS

M. A. PERSINGER*, L. L. COOK and S. A. KOREN

*Behavioral Neuroscience Laboratory, Laurentian University, Sudbury, Ontario, Canada*

*(Received in final form 26 March 1999)*

After inoculation with spinal cord and complete Freund's adjuvant, female Lewis rats were exposed to weak, 7 Hz complex magnetic fields or to the control condition. The computer-generated magnetic field, whose amplitude varied from 15 nT to 60 nT every 6 to 12 sec, was presented for 6 min every hour between midnight and 0800 of the scotophase. In two replicates of the experiment, the rats exposed to the field displayed statistically significant suppression relative to sham-field controls (effect size = 55%) of the overt symptoms of experimental allergic encephalomyelitis which included hindleg paralyses.

*Keywords:* Extremely low frequency magnetic fields; rats; experimental multiple sclerosis; nocturnal exposures; nanoTesla amplitudes

The exacerbation and the attenuation of the symptoms of multiple sclerosis (MS) have been associated with seasonal and geomagnetic variables (Sandyk, 1992a). Stimuli that evoke immunosuppression, which include pregnancy or the administration of synthetic corticosteroids such as prednisolone (prednisone), are known to suppress the overt symptoms and to delay their recurrence (Matthews, 1991). The "pulsatile" presentation of prednisolone has been shown to be more effective to ameliorate these symptoms than chronic, low-level administration (Weiner *et al.*, 1993).

*Address for correspondence: Behavioral Neuroscience Laboratory, Laurentian University, Sudbury, Ontario, P3E 2C6. Fax: (705)-671-3844, e-mail: MPersinger@admin.laurentian.ca

Experimental allergic encephalomyelitis (EAE) has been employed as an animal model that displays characteristics very similar to MS (Alford, Kies and Suckling, 1984). We have shown that singular, traumatic insults to the brain, such as inductions of seizures by a single systemic injection of lithium and pilocarpine, can either amplify or suppress the severity of the overt symptoms depending on the time between the inoculation and the induction of the seizures (Missaghi, Richards and Persinger, 1992). There is strong evidence that the modulation of the severity of the symptoms is mediated by the corticotrophin releasing factor (CRF)-ACTH-cortisol (corticosterone) system. The release of ACTH during limbic electrical seizures is related to the duration of afterdischarges. Durations of afterdischarges less than 10 sec decreased while afterdischarges longer than 10 sec increased the release of ACTH (Gallager *et al.*, 1987).

Recently we (Michon and Persinger, 1997) have shown that nocturnal seizures within a population of male limbic epileptic rats increased significantly when they were exposed to a 7 Hz magnetic field whose amplitudes incrementally increased or decreased over a 5 min period from the minimal value of 15 nT to a peak of 60 nT. The effect size (equivalent to the coefficient of determination or $r$-squared value) for the numbers of seizures was similar to that associated with the occurrence of average daily geomagnetic activity over 40 nT. In another study (Persinger, 1996), limbic epileptic rats exposed for 6 min once per hour every hour during the night cycle to an applied 7 Hz field whose amplitude shifted every 30 sec from 10 nT to 80 nT displayed increased numbers of overt seizures to daily food presentations (even though the rats were maintained on food *ad libitum*) if the presentations were presented within one hour before the onset of the scotophase (Persinger, 1995).

The general hypothesis has been that the suppression of nocturnal melatonin (Persinger, 1995) by specific temporal structures of complex magnetic fields (Reiter, 1993; Yaga *et al.*, 1993) that simulate the amplitude characteristics of sudden storm commencements and other geomagnetic phenomena should disinhibit (release) CRF within the limbic region and hence lower the threshold for seizures (Zeise and Semm, 1985). We reasoned that such a release in CRF by suppression of this seasonally varying (Bartsch *et al.*, 1994) serotonin derivative should also facilitate immunosuppression in rats sensitized to spinal cord. We predicted that the severity of the overt (clinical) symptoms to EAE should be significantly reduced or eliminated.

## METHOD

### Subjects

In two separate blocks of experiments, a total of 16 (8 per experiment), 120 day old Lewis female albino rats, obtained from Charles River, were employed as subjects. Females of this age range rather than males were employed because of the former's greater reliability for the development of EAE by our methods. The subjects had been habituated to the colony conditions in a room other than the experimental room for at least one month. The light : dark cycle had been 12 : 12 and the controlled ambient temperature was 20 deg C.

### Exposure Procedure

Each rat in a block ($n = 8$) was injected with 0.2 cc of an homogenized mixture of fresh spinal cord from a female Lewis rat and Freund's complete adjuvant; 0.1 cc was injected in the posterior section of each hindfoot pad. This method: 1) reduces the duration of post-inoculation edema within the pads such that the rats display normal ambulation within 48 hr, and, 2) the average severity of the symptoms is within the midrange of our measurement scale such that either the immunofacilitation or immunosuppression of treatments can be assessed equally. Both this procedure, because it resulted in the least duration of hindfoot hypertrophy (compared to one hindfoot pad), and the experiment had been approved by the university's Animal Care Committee.

After the injection, pairs of female rats were placed in each of the cages within the coils and in each of the controls cages beneath the coils (2 rats per cage $\times$ 4 cages = 8 rats per experiment). The cages contained wood shavings in order to minimize distress during ambulation. Food and water were available *ad libitum*. The first exposure to the experimental field occurred within 12 hr after the inoculation. The presence of the field was verified directly by the first experimenter every second night during the initial presentation (midnight) and more intermittently during the latter periods. In addition, the computer recorded the hourly activation of the fields (later examination of the diskettes verified activation of the field for 6 min, 8 times per night, every night during the experiments).

Entry into the exposure area during the scotophase did not exceed 10 min, once per night, and was luminated by one overhead red fluorescent lamp (1 lux) which has been shown not to interfere with the seizure displays or general activity of epileptic rats exposed to weak, complex magnetic fields (Michon and Persinger, 1997). Food and water were refreshed during these nocturnal periods once every three to four days. The wood shavings (bedding) were replaced every seven days during the light period. The experiments were completed during July and August of 1996.

## Clinical Measures

Beginning on post-inoculation day 10 and for 6 successive days, each rat was assessed singly and daily during the mid-photophase to discern the severity of the symptoms. During this post-inoculation period rats always display the symptoms if the syndrome is going to develop (Missaghi, Richards and Persinger, 1992). They were ranked by an experienced rater who was not familiar with the hypothesis on a 0 to 4 scale, modified after Missaghi, Richards and Persinger, (1992) that included: 0: normal, 1: loss of tail tonicity, 2: ataxia, 3: partial analysis of both hind legs, 4: complete hind leg paralysis and loss of bladder control. The interrater reliability for this scale has been greater than $r = .90$ for more than 100 animals.

## Exposure System

The magnetic fields were amplitude modulated by a personal (Commadore) computer. The output was coupled to two (32 cm × 36 cm by 45 cm) 30 Ohm coils separated by 5 cm. Four identical 28 cm by 34 cm by 45 cm plastic cages with plastic grid tops were employed to house the rats. One cage was placed within each of the two coils and within each of the two controls positions immediately below the two coils. The distance between the bottom of a coil and the top of the most proximal edge of a control cage was 15 cm.

Lighting within the four cages was similar, was supplied by overhead fluorescent lamps, and was between 700 and 800 lux (Gossen Panlux meter) during the day phase. The light : dark cycle was 12 : 12 and light onset was 0800 hrs. The long axes of all four cages were oriented at a 15 deg angle to the east of the N-S geomagnetic direction. According to the fluxgate (Scintrex MF-2) magnetometer, the natural (geomagnetic) vertical static field (no experimental fields activated) ranged between 32,000 nT and 36,000 nT.

The programming of the Commodore computer was determined to activate a Model EU 81A Heath-Schlumberger function generator (output attenuated by a factor of 10) for 6 min once every hour, beginning at midnight until 0800 hours. The temporal structure of the changes in amplitude and the duration of this structure was designed to simulate some of the characteristics of a sudden impulse or sudden storm commencement (rise times between 5 min and 6 min) within the geomagnetic field as reported by Mayaud (1975). As can be seen in Figure 1, there were 30 shifts in amplitude during the 360 sec presentation. The time between shifts (increase or decrease) in amplitudes during the first half was 12 sec while the time between shifts in amplitudes for the second half was 6 sec. These temporal shifts were considered similar to the periods of excursions in amplitudes as geomagnetic activity increases during unsettled to active times.

The ("ripple") frequency of the variable amplitudes, supplied by the function generator, was a 7 Hz bipolar square wave. The intensity was set such that the minimum amplitude was 15 nT and the maximum amplitude was 60 nT, as measured by a Metex M-3800 digital multimeter and sensor [Electric Field Measurement, W. Stockbridge, MA 01266], within the center of the each coil and parallel with its long axis. The field strength along the long ($z$) axis of the coil within the housing area decreased by 40% to the outer edge, producing a gradient during the maximum strength (60 nT) of about .1 nT/cm. The maximum central field strengths within the two cages did not differ by more than 5 nT. Field strengths along the $x$ and $y$ axes were 10 nT or less.

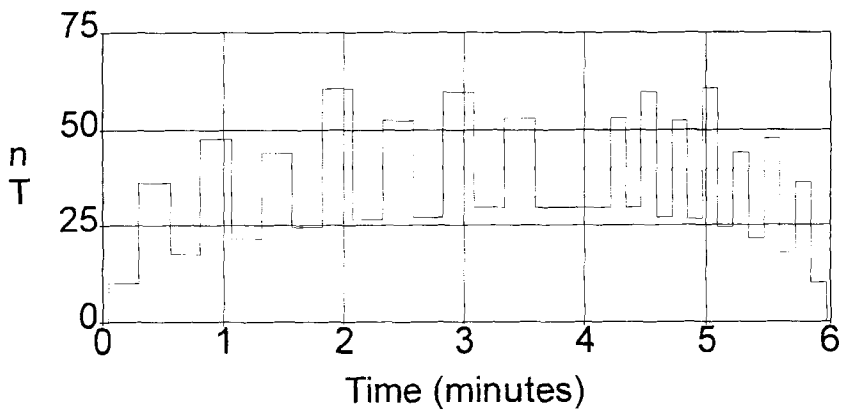


FIGURE 1 Morphology of the 6 min, amplitude shifting (in nT), 7 Hz field that was presented once per hour for 8 hr after midnight (dark cycle beginning at 2000 hrs) after the inoculation with emulsified spinal cord and complete Freund's agent.

Within the control cages, immediately below each coil, field strengths within the $x$, $y$ and $z$ axes were 10 nT or less and did not change when the experimental fields were activated. The background 60 Hz field within the exposure regions for all four cages was 10 nT (0.1 mG) which was the limit of the sensor and multimeter. Baseline studies, to insure there was no *a priori* bias for developing EAE in any of the four positions (two coil, two control), had shown that the mean and standard deviation for the clinical scores for groups of rats exposed to the four positions did not differ significantly (grand mean = 1.8, SD = 1.0).

### Statistical Analyses

The clinical scores for each day for each rat for each condition (reference group *vs.* magnetic field stimulation) for the two experiments were analyzed by three way analyses of variance with one level repeated (days) and two not repeated (treatment; block). *Post hoc* analyses were also completed. All analyses involved SPSS software.

## RESULTS

The three way analysis of variance with one level repeated (daily clinical scores) and two not repeated (no field *vs.* field and Experiment I *vs.* Experiment II) demonstrated that the rats exposed to the amplitude modulated 7 Hz magnetic field, presented for 6 min once per hour during the scotophase, displayed statistically significant reductions [$F(1, 12) = 15.29$, $p < .01$] in the overt symptoms of experimental allergic encephalomyelitis relative to the non-exposed controls. The treatment was conspicuous qualitatively, even by casual observers, and accounted for 55% of the variation in clinical scores between the two groups (Fig. 2). The mean and the standard deviation for the three days of observation after the onset of the clinical symptoms (day 13) for the group exposed to the complex magnetic field patterns were 0.2 and 0.4, respectively while these values for the controls were 2.0 and 1.1, respectively.

There was no significant difference between the experiments [$F(1, 12) = 0.55$, $p > .05$] nor an interaction between experiments and either the treatment [$F(1, 12) = 0.87$, $P > .05$] or the repeated measures [$F(2, 24) = 0.12$, $p > .05$]. There was a statistically significant interaction between the days of observation and the exposure to the fields [$F(2, 24) = 13.32$, $p < .001$; partial

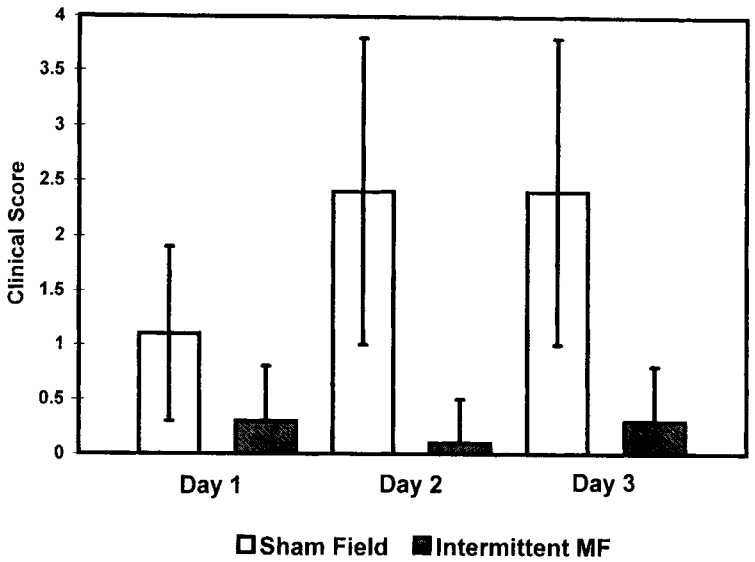


FIGURE 2 The severity of symptoms of experimental allergic encephalomyelitis (vertical axis; see text for definition) for rats that were exposed either to the magnetic field (Fig. 1) or to control conditions for the first three days after onset (day 13 after inoculation) of the first clinical symptoms. Vertical bars indicated plus/minus 1 standard deviation.

eta-squared = 53%] which was also primarily responsible for the difference between days [$F(2, 24) = 10.92$, $p < .001$]. *Post hoc* analyses (paired $t$-tests comparing days for each the field and no field groups separately) indicated that the primary source of the interaction was the absence of change of the clinical signs of the rats exposed to the experimental fields over the observation period compared to the successive worsening of the clinical signs for the control or reference group. The three way interaction was not statistically significant [$F < 1.00$].

## DISCUSSION

In two separate experiments, whose results did not differ significantly, female Lewis rats that had been inoculated with spinal cord and complete Freund's adjuvant and then exposed to weak (nT range) amplitude-modulated, 7 Hz magnetic fields presented during the night cycle displayed either a marked reduction or suppression of the overt symptoms of experimental allergic encephalomyelitis (EAE) relative to controls. The equivalent correlation coefficient for the treatment effect was 0.74 and indicated that

55% of the variance in the clinical symptoms between the two groups was accommodated by the treatment. The absence of significant differences between the experiments suggests the consistency of the effect.

Although we did not address the question of whether the field treatment suppressed the overt symptoms or simply delayed the onset (to a similar severity) of the symptoms, subsequent unpublished experiments by Lisa Cook have indicated the former. When rats were allowed to complete the entire course of the disease, we found no evidence of a delayed exacerbation of the symptoms in the field exposed rats. Whether or not this robust clinical effect is also reflected as a reduction of neuropathological indicators, such as infiltrations of mononuclear cells into the spinal cord and lower brain stem, must still be evaluated.

The results are consistent with our hypotheses that: (1) weak, complex magnetic fields whose temporal structure and secondary (ripple) frequencies simulate geomagnetic activity can affect immunological processes and (2) the effects of weak magnetic fields may involve suppression of nocturnal melatonin levels and enhanced excitability of limbic neurons and/or the release of corticotrophin releasing factor (Persinger, 1996) which encourages immunosuppression. Our pilot studies have indicated that bursts of higher intensity fields (1000 nT) once every four seconds for several hours during the day did not ameliorate the symptoms ($M$ and SD for clinical signs = 1.4 and 1.3, respectively). Other rat preparations have also displayed sensitivity to weak nocturnally applied magnetic fields. Michon and Persinger (1997) who measured (videotaped) the spontaneous display of overt seizures in epileptic rats between 0200 to 0400 hrs, reported that the presentation of complex magnetic fields whose strength exceeded 40 nT increased the incidence of seizures. Those rats were housed singly in different Helmholtz coils (driven by different equipment) than the ones employed in the present study.

The interstimulus interval for the presentation of the envelope is considered an important variable for the effect of weak magnetic fields. In the present experiment, the interstimulus interval was 54 min while the duration of the envelope was 6 min. Stated alternatively, the envelope was presented for 1 unit every 10 units of time. The importance and precision of the temporal parameters in the pulsatile release of hormones are well known in endocrinology. Interestingly, one of the most recent methods to treat MS, the human analogue of EAE, has been the intermittent injection of the immunosuppressor cyclophosphamide (Weiner *et al.*, 1993).

The efficacy of the morphology of bioeffective complex, magnetic fields may contain several components that are analogous to a polynomial

equation. For example, Litovitz *et al.* (1997) have reported two components in the temporal sensing of electromagnetic fields as inferred by an increase in ornithine decarboxylase. They found two time constants, approximately 100 msec and 10 sec, that were involved with the biological detection of extremely low frequency electromagnetic fields. Interestingly, in the present study the shift in amplitudes of the 7 Hz ripple frequency occurred between 6 sec and 12 sec.

Sandyk (1992b) has reported that very weak, (*i.e.*, 7.5 picotesla flux density) magnetic fields (frequency of stimulation between 2 Hz and 7 Hz) can improve symptoms of multiple sclerosis in humans. He has also shown experimentally the potential relationship between pineal melatonin activity and the occurrence of multiple sclerotis symptoms. Whereas injection of melatonin worsened the symptoms of MS, immediate application of weak complex magnetic fields reduced the symptoms. Sandyk also emphasized that elevations of corticosteroids, which are often clinically induced to accommodate the acute exacerbations of MS, inhibit melatonin secretion.

The effectiveness of our experimental extremely low frequency, amplitude-modulated (nanoTesla range) magnetic fields for reducing the symptoms of EAE indicates that a re-examination of epidemiological data for MS may be in order. We suggest that the positive association between MS and geomagnetic latitude may reflect an optimal ratio of the average time-varying component of geomagnetic activity (within the 10 to 100 nT range) with respect to the steady-state magnetic field (within the 50,000 nT range). If such "Weber fractions" (Persinger, 1997) exist, then enhanced occurrence of MS within mid-latitudes may reflect an environmental factor that facilitates the primary process. However if the results of this study are generalizable, geomagnetic latitudes in which there is a very low incidence of MS might be associated with a ratio that actively suppresses the occurrence.

### *References*

Alford, E. C., Kies, M. W. and Suckling, A. J. (Eds.) (1984) *Experimental allergic encephalomyelitis: a useful model for multiple sclerosis.* New York: A. R. Liss.

Bartsch, H., Bartsch, C., Mecke, D. & Lippert, T. H. (1994) Seasonality of pineal melatonin production in the rat: possible synchronization by the geomagnetic field. *Chronobiology International*, **11**, 21–26.

Gallagher, B. B., Flanigin, H. F., King, D. W., Murro, A. M. & Littleton, W. (1987) ACTH secretion in human temporal lobe epilepsy. In: Engel, J. (Ed): *Fundamental mechanisms of human brain function.* (pp. 201–207) New York: Raven.

Litovitz, T. A., Penafiel, M., Krause, D., Zhang, D. & Mullins, J. M. (1997) The role of temporal sensing in bioelectromagnetic effects. *Bioelectromagnetics*, **18**, 388–395.

Mayaud, P. N. (1975) Analysis of storm sudden commencements for the years 1868–1967. *Journal of Geophysical Research*, **80**, 111–122.

Matthews, W. B. (1991) *McAlpine's multiple sclerosis*. (pp. 109–112, 257–261) New York: Church-Livingstone.

Michon, A. & Persinger, M. A. (1997) Experimental simulation of the effects of increased geomagnetic activity upon nocturnal seizures in epileptic rats. *Neuroscience Letters*, **224**, 53–56.

Missaghi, B., Richards, P. M. & Persinger, M. A. (1992) Severity of experimental allergic encephalomyelitis in rats depends upon the temporal contiguity between limbic seizures and inoculation. *Pharmacology, Biochemistry and Behavior*, **43**, 1081–1086.

Persinger, M. A. (1995) Geophysical variables and behavior: LXXIX. Overt limbic seizures are associated with concurrent and premidscotophase geomagnetic activity: synchronization by prenocturnal feeding. *Perceptual and Motor Skills*, **81**, 83–93.

Persinger, M. A. (1996) Enhancement of limbic seizures by nocturnal application of experimental magnetic fields that simulate the magnitude and morphology of increases in geomagnetic activity. *International Journal of Neuroscience*, **86**, 271–280.

Persinger, M. A. (1997) Metaphors for the effects of weak, sequentially complex magnetic fields. *Perceptual and Motor Skills*, **85**, 204–206.

Reiter, R. J. (1993) Static and extremely low frequency electromagnetic field exposure: reported effects on circadian production of melatonin. *Journal of Cellular Biochemistry*, **51**, 394–403.

Sandyk, R. (1992a) The pineal gland and clinical course of multiple sclerosis. *International Journal of Neuroscience*, **63**, 206–215.

Sandyk, R. (1992b) Successful treatment of multiple sclerosis with magnetic fields. *International Journal of Neuroscience*, **66**, 237–250.

Weiner, H. L., Mackin, G. A., Orav, E. J., Hafler, D. A., Dawson, D. M., LaPierre, Y., Herndon, R., Lehrich, J. R., Hauser, S. L., Turel, A., Fisher, M., Birnbaum, G., McArthur, J., Butler, R., Moore, M., Sigsbee, B. & Safran, A. (1993) Intermittent cyclophosphamide pulse therapy in progressive multiple sclerosis: final report of the Northeast cooperative multiple sclerosis treatment group. *Neurology*, **43**, 910–919.

Yaga, K., Reiter, R. J., Manchester, L. C., Nieves, H., Sun, J.-H. & Chen, L.-D. (1993) Pineal sensitivity to pulsed static magnetic field changes during the photoperiod. *Brain Research Bulletin*, **30**, 153–156.

Zeise, M. L. & Semm, P. (1985) Melatonin lowers excitability of guinea pig hippocampus neurons *in vitro*. *Journal of Comparative Physiology*, **157**, 23–29.